Do forest refugia and riverine barriers promote genetic diversity among species in the *Hybomys* division?

Research Thesis

Presented in partial fulfillment of the requirements

for graduation with Research Distinction in Biology

in the Undergraduate Colleges of The Ohio State University

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The Ohio State University

April 2019

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ABSTRACT

The West African tropical rainforest is an ecosystem rich in biodiversity in a number of forest-dwelling mammals. We examined the role of both forest fragmentation during the Pleistocene and rivers acting as physical barriers in influencing diversification. The aim of this study is to investigate how these geographical barriers in Western Guinea lowland forest (WGLF) and forest fragmentation events affect the relationship within species of murid rodents known as the *Hybomys* division. We included samples from all genera in the *Hybomys* division with all West African species represented. More specifically, the species being researched are two species distributed across forest in West Africa from the genus *Typomys (*the Liberian striped mouse, *T. planifrons* and Temminck's striped mouse, *T. trivirgatus)* and the single species in the genus *Dephomys* (the defua rat, *Dephomys defua*). In this study a combination of mitochondrial (cytochrome *b*, *Cytb*) and nuclear (Interphotoreceptor Retinoid Binding Protein, *Rbp3*) data were used to generate a molecular phylogeny. Our results showed latitudinal patterns between Guinea and Côte d'Ivoire, supporting the two small forest refugia hypothesis. This latitudinal separation in *D. defua* diverged approximately 1.36 Mya following the aridity event from 1.8-1.6 Mya. *Typomys trivirgatus* diverged about 0.88 Mya aligned with the aridity event from 1.0-0.8 Mya. The pattern in *T. planifrons* is not as clear because the Guinea samples were not monophyletic. *Typomys planifrons* from Guinea and Côte d'Ivoire diverged about 0.67 Mya, shortly after the aridity event from 1.0-0.80 Mya It is worth noting that the insufficient sample size and limited sample distribution could affect our ability to detect patterns. In the WGLF, however, past forest refugia appear to have had a greater impact on populations compared to the Cavally River.

INTRODUCTION

The increase in biodiversity from polar to tropical regions is one of the most fundamental patterns in biology (Willig et al. 2003). Located at latitudes surrounding the equator, the tropical rain forest, is the most biologically diverse terrestrial biome (Eiserhardt et al. 2017). The West African rain forest is currently one of the largest blocks of rainforest on earth (Nicolas et al. 2019). The Guinean Forests of West African have specifically been noted as one of the most biologically diverse regions in the world (Ceballos and Ehrlich, 2006). Based on the presence of the Dahomey Gap, a 200-km-wide forest savanna mosaic, the forest can be further divided into two sub-regions: Upper Guinea (UG) and Nigeria-Cameroon (Demenou et al. 2016;White 1979). The UG sub-region covers from southern Guinea and through Sierra Leone, Liberia, Côte d'Ivoire and Ghana, and then into parts of Togo (Lebbie 2019). Within the UG sub-region, west of the Sassandra River, lies the Western Guinea lowland forest (WGLF) (Lebbie 2019). There are many hypotheses that could be contributing to the high level in organismal diversity in the UG lowland forest, including the following two: The 'Pleistocene refuge' and riverine barrier hypotheses.

The 'Pleistocene refuge' hypothesis emphasizes the expansion and contractions of forested habitats due to large scale climate shifts (Haffer 1982). Paleoclimatic changes have been supported by paleontological records identifying that there have been major ecological and organismal changes across Africa due to alternating climate intervals of wet and dry conditions (deMenocal 2004). Records of African fauna and weather suggest there were three shifts toward drier and more fragmented forest: 2.9-2.4 Ma, 1.8-1.6 Ma and 1.2-0.8 Ma (deMenocal 2004). These prolonged periods of aridity correspond with forest fragmentation, driving allopatric diversification among forest-dwelling mammals (Jacquet et al. 2014; Nicolas et al. 2008).

Next, the 'riverine barrier' hypothesis highlights the effects that rivers have when acting as physical barriers affecting the distribution of mammals (Jacquet et al. 2014; Nicolas et al. 2010). In Africa, rivers have been previously noted as an influencer for intraspecific genetic diversification within rodents (Nicolas et al. 2019). Therefore, molecular data can be used to test how genetic variation is structured across rivers within the WGLF. The two hypotheses are not mutually exclusive; both of these hypotheses have been suggested as the main drivers in diversification among forest-dwelling genera (Bohoussou et al. 2015).

Rodents are the ideal organism to use when investigating phylogeogaphic patterns because of their short life-span, limited dispersal ability, and their close association with their habitat (Fedorov et al. 2008). There is an increasing volume of phylogeographic studies on rodents across Africa; however, a limited number have focused on WGLF rodents (Bohoussou et al. 2015; Jacquet et al. 2014; Nicolas et al. 2008).

Murid rodents make up approximately 155 genera and have greater than 800 recognized species, making Muridae the most diverse mammalian family (Musser and Carleton 2005; Wilson et al. 2017). The subfamily Murinae can be found in the Old World and has a distribution across the entire continent of Africa (Lecompte et al. 2008). As a result of the immense number of species, previous studies have been making classifications at a tribal level (Lecompte et al. 2008). In Africa, a monophyletic group of grass rats and their forest dwelling relatives form the tribe Arvicanthini (Ducroz et al. 2001). Beyond that, Musser and Carleton (2005) employed a less formal arrangement of genera. The tropical rainforests of West Africa are home to the *Hybomys* division which has recently been divided into 4 genera: *Hybomys, Dephomys, Stochomys* and *Typomys* (Missoup et al., 2018; Pradhan et al. in review). In this project, I focused on two species in the genus *Typomys (*the Liberian striped mouse, *T. planifrons* and

Temminck's striped mouse, *T. trivirgatus)* and the single species in the genus *Dephomys* (the defua rat, *Dephomys defua*). It is unclear where the major biogeographical breaks dividing populations within these and similar species are located; however, with the use of genetic data collected from these assorted species the barriers driving diversification can be made clear.

Currently there are two proposed models of how the forest was shaped at the Last Glacial Maxima (LGM). Maley (1996) proposed two distinct refugia throughout the WGLF, separating forest blocks in Guinea and parts of Liberia and Côte d'Ivoire. In contrast Anhuf et al. (2006) proposed, one large forested region covering much of West Africa at the LGM. Few maps have been presented for earlier glacial maxima; therefore will assume that the proposed forest refugia from the LGM represents earlier aridity events of the Pleistocene (Dupont et al. 2000: Bohoussou et al. 2015). In this study, I used a combination of mitochondrial (Cytochrome *b*, *Cytb*) and nuclear (Interphotoreceptor Retinoid Binding Protein, *Rbp3*) data to generate a molecular phylogeny within species in the *Hybomys* division in order to test between these competing hypotheses. This study aims to (i) identify any genetic variation within species in the *Hybomys* division; (ii) recognize any biogeographic barriers or breaks within these species that can be attributed to a river barrier; and (iii) compare how our results compare to proposed forest refugia and large scale climate shifts.

MATERIALS AND METHODS

DNA extraction, sequencing, and alignment. — Prior to this study, several expeditions to West Africa were conducted to obtain specimens that were used in this study (Alonso et al. 2005; Decher et al. 2010, 2013, 2015; Norris 2006). Kidney and Liver tissues from the expedition were stored at room temperature in 95% ethanol solution. DNA extraction was carried out from representatives of *T. trivirgatus, T. planifrons* and *D. defua* (Fig. 1). Before the DNA extraction,

tissues were soaked in distilled water for 10 minutes to remove ethanol and increase output (Kilpatrick 2002). Kidney and Liver tissue were sliced into smaller pieces and incubated overnight at 56°C in 180µL of ATL tissue lysis buffer and 20µL of proteinase K. The DNA extraction was carried out using the Gentra Puregene Mouse Tail Kit (QIAGEN, Germantown, MD) once all the cells were lysed.

The *Cytb* gene fragments were amplified by using polymerase chain reaction (PCR) with specifications for denaturing, annealing and extension: 35 cycles of 94°C (1 min), 50°C for (1 min), and 72°C (1 min) (Norris et al. 2008; Saiki et al. 1988). The mitochondrial *Cytb* gene was sequenced in fragments with primer pairs CytbA and CytbE, Cytb A and 752R, and CytbD and END2 (Norris 2009; Schenk et al. 2013; Tiemann-Boege et al. 2000). The *Rbp3* gene was sequenced with the primers IRBP119A2 and IRBP878F (Schenk et al. 2013). All additional sequences in this study were obtained from GenBank (Table 1). The products from our PCR were sequenced with the same primers offsite at the TACGen Sanger sequencing facility (Richmond, California) and The University of Vermont DNA Analysis Center. Sequences were aligned by eye and compared to DNA sequences found on GenBank.

Phylogenetic analyses.— The final alignments for the *Cytb* gene included 1140 bases and 39 taxa, and for the *Rbp3* gene included 1236 bases and 28 taxa (Table 2). Bayesian analyses were performed using BEAST 1.8.4 (Drummond and Rambaut 2007). In order to select the best-fit models of nucleotide substitution, I used jModeltest 2.1.7 (Darriba et al. 2012) which selected GTR + I + gamma model of substitution for *Cytb* and GTR+ gamma for *Rbp3*. I partitioned the analysis by gene. In order to estimate divergence dates on the tree, I calibrated at the Otomyini – Arvicanthini divergence (95% 8.7 to 10.1; Kimura et al. 2015). I fixed the root of the tree by applying a lognormal prior where zero offset = 8.7, mean = -0.695, and stdev = 0.6308. The

BEAST analysis was run for 100,000,000 generations, with trees sampled every 10,000 generations. The BEAST run was visualized in Tracer v1.7.1 (Rambaut et al 2014) to verify burnin. The maximum clade credibility tree was constructed in TreeAnnotator (Drummond and Rambaut 2007) using a burnin of 10,000 trees (Fig. 2).

RESULTS

Our results showed Arvicanthini and Otomyini diverged 9.18 (95% highest posterior density 10.0-8.79) Mya, consistent with the calibration (Fig. 2). The 4 genera within the *Hybomys* division form a monophyletic group, and our data weakly support a sister relationship with all members of the Arvicanthini except for *Oenomys* and *Golunda* (posterior probability value (PP) = 0.73) (Fig. 2). These two clades diverged 7.84 (95% 9.30- 6.30) Mya. The two *Typomys* species (*T. planifrons* and *T. trivirgatus*) are monophyletic (PP=1) and are sister to a well-supported (PP=1) clade consisting of the remaining 3 genera in the *Hybomys* division at 6.84 (95% 8.35-5.13) Mya (Fig. 2). The two species of *Typomys* last shared a common ancestor 3.97 (95% 5.50-2.58, PP=1) Mya. The single *Hybomys* species (*H. univittatus*) in our sample is sister to a *Stochomys+Dephomys* clade (PP=0.93) which diverged 6.65 (7.75-4.40) Mya (Fig. 2).

Two of the three focal species in this study displayed separation between Guinea and Côte d'Ivoire. *Dephomys defua* from Guinea diverged from Ivorian samples 1.36 (95% 2.31-0.76) Mya, and *T. trivirgatus* diverged 0.88 (95% 1.57-0.43) Mya (Fig. 2). The pattern in *T. planifrons* is not as clear because the Guinea samples were not monophyletic. The *T. planifrons* samples from Côte d'Ivoire diverged 0.67 (95% 1.57-0.43) Mya from their closest relatives in Guinea (Fig. 2). Specimens from *D. defua* in Côte d'Ivoire were paraphyletic with Liberia samples, diverging at 0.33 (95% 0.74-0.09) Mya (Fig. 2). Our results show integrations of *T. planifrons* from Sierra Leone with samples from Guinea, which suggests that Sierra Leonean

populations may have been the result of migration from Guinea around 0.26 (95% 0.52-0.1) Mya.

DISCUSSION

The Tribe Arvicanthini was molecularly defined with 2 genes and included 7 genera with strong support (PP=1), similar to the findings of Lecompte et al. (2008). Bayesian analysis offered strong support (PP=1) for monophyly of the *Hybomys* division, but its position within the Arvicanthini is poorly resolved. I recovered the *Hybomys* division as sister to all Arvicanthini in the dataset except for *Oenomys* and *Golunda*, but with only weak support (PP = 0.71). The *Hybomys* division is clearly nested within an African radiation. Within the *Hybomys* division, my molecular analysis recovered the four genera as monophyletic (PP = 1 except for *Hybomys*, which contained a single sample). As shown by Missoup et al. (2018) and Pradhan et al. (in review), *Typomys* represents the basal lineage to the other 3 genera. In agreement with past molecular studies (Pradhan et al. in review; Missoup et al. 2018; Steppan and Schenk 2017), our results showed strong support for *Hybomys univittatus* being a sister group (PP= 0.93) to *Dephomys* and *Stochomys*. The grouping of *Dephomys* and *Stochomys* was well supported in this study (PP=1). All three species found in UG formed separate clades that were monophyletic (PP = 1). *Typomys* may have originated in UG, but the closest relatives to *Dephomys* are central African.

According to Maley (1996) two forest refugia (one in SE Guinea and another in Liberia + SW Côte d'Ivoire) were present during glacial maxima in the WGLF. My results in *D. defua* support this hypothesis as two subclades are recovered: one restricted to SE Guinea (IG; PP=1) and one group restricted to Liberia + SW Côte d'Ivoire (L+IC; PP=1) (Fig. 2). The divergence of these two subclades (1.36; 95% HPD= 2.3-0.8 Mya) falls between two aridity events proposed

by deMencol (2004), one occurring 1.8-1.6 Mya and the other at 1.0-0.8 Mya. A similar phylogeographical pattern was identified in *T. trivirgatus* (~ 0.90 Mya), which aligns with the aridity event occurring 1.0-0.8 Mya. Prolonged periods of aridity in Africa are thought to have started around 1 Mya (deMenocal 2004). The genetic divergence between SE Guinea and SW Côte d'Ivoire has been found in another rodent (Bohoussou et al. 2015). Bohoussou et al. (2015) found high genetic diversity within an African swamp rat species, *Malacomys edwardsi*, including evolutionary divergence that matched the same two refugia hypothesis presented by Maley (1996). *Malacomys edwardsi* samples from Mount Nimba (Guinea) formed a sister group to *M. edwardsi* samples collected from the SW Côte d'Ivoire. This divergence is estimated to have occurred after 0.5 Mya (Bohoussou et al. 2015).

The pattern in *T. planifrons* is not as clear because the Guinea samples were not monophyletic (CI+III). Similar to *T. planifrons*, other studies have not found high genetic divergence between these two areas for other rodent and shrew species (Jacquet et al. 2014; Nicolas et al 2008, 2019). Jacquet et al. (2014) worked on a forest dwelling shrew, *Crocidura obscurior*, which did not show separation between SE Guinea and SW Côte d'Ivoire. Contrary to *D. defua* and *T. trivirgatus*, *Praomys rostratus* is a generalist species that is more resilient to forest disturbance and is understood to have been able to cross savanna habitat (Nicolas et al. 2008). Additional information on the ecological behavior of *D. defua* is required to have a complete understanding on how forest fragmentation affects their dispersal. Currently, it is understood that *D. defua* may be partly arboreal (Happold 2013; Wilson et al. 2017).

Findings from this study suggest that the Cavally River is not a complete barrier for *D. defua*, because the Côte d'Ivoire samples were paraphyletic with respect to the Liberia sample. Similar to our results, there appears to be gene flow for some other small mammals such as the

West African pygmy shrew (Jacquet et al. 2014) and *Praomys* (Nicolas et al. 2008). For example, *P. rostratus* found in SW Côte d'Ivoire do not exhibit much difference from populations across the Cavally River (Nicolas et al. 2008). Climate variation plays a major role in the size, location and direction of the river; some major aridity events could even cause rivers to completely disappear resulting in regional mixing (John 1986). Additional information on the historical structure and role of the Cavally River are needed to help determine its effects on allopatric speciation.

Our results show integrations of *T. planifrons* from Sierra Leone with samples from Guinea (SL+III), which suggests that Sierra Leonean populations may have been the result of migration from Guinea around 0.26 Mya (PP=1), which is after the most recent aridity event. These results have a similar pattern to those found in *M. edwardsi,* which showed gene flow between samples in Guinea and Sierra Leone (Bohoussou et al. 2015). Expansion of vegetation and forest dwelling fauna occurs between aridity events, allowing for migration between forest blocks (deMenocal 2004). One species which does show differentiation between SE Guinea and Sierra Leone is *P. rostratus* (Nicolas et al. 2008). This species is found in more marginal forest habitat than members of the *Hybomys* division (Happold 2013; Nicolas et al. 2008). Perhaps *Praomys* persisted in poor quality forest in the Sierra Leonean region during glacial cycles, whereas *Dephomys, Typomys,* and *Malacomys* were locally extirpated and subsequently recolonized from Guinea.

Our results showed latitudinal patterns between Guinea and Côte d'Ivoire, supporting the two small forest refugia hypothesis. This latitudinal separation in *D. defua* and *T. trivirgatus* align with Maley's (1996) proposed two forest refugia model. In addition, our results showed no longitudinal patterns among the species in Liberia and Côte d'Ivoire. There was no supporting

evidence found for the Cavally River acting as an allopatric barrier for *D. defua*. In addition, integration of samples from the Sierra Leone forests with Guinea support post glacial migratory patterns from Guinea. It is worth noting that the insufficient sample size and lack of sample distribution could affect our ability to detect patterns. Future research could be improved by sampling more individuals throughout localities across the WGLF.

Although in my study the Cavally River did not restrict the dispersal of the *Hybomys* division, other studies have demostrated that rivers can act as complete physical barriers (Bohoussou et al. 2015; Jacquet et al. 2014; Nicolas et al 2008, 2011). River systems throughout the UG forests that have been identified as barriers to dispersal in small mammals including the Volta, Sassandra, and Comoé rivers (Bohoussou et al. 2015; Jacquet et al. 2014; Nicolas et al 2008, 2019). In the WGLF, however, past forest refugia appear to have had a greater impact in populations compared to the Cavally River.

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FIGURE LEGENDS

Fig. 1.- Distribution of samples used in this study. Species are differentiated by shape and location is differentiated by color. Triangles= *D. defua*, Circles= *T. planifrons*, and Squares=*T. trivirgatus*. The two competing hypotheses for the reconstructed WGLF forest cover are labeled: I and II. **I.** The transparent area represents the single forest refugium presented by Anhuf et al. (2006), **II.** The shaded areas represent the two smaller refugia modeled by Maley (1996).

Fig. 2.- Bayesian tree showing relationship and divergence times. Boxes at nodes represent 95% HPD around age estimates and the large red circles represent high posterior probability values, and major aridity events are highlighted in orange. The tree was calibrated using the Otomyini – Arvicanthini divergence (95% 8.7 to 10.1; Kimura et al. 2015). Clades are grouped by species and locality: IG= *D. defua* (Guinea), IC=*D. defua* (Côte d'Ivoire), L= *D.defua* (Liberia), IIG= *T. trivirgatus* (Guinea), IIC= *T. trivirgatus* (Côte d'Ivoire), III= *T. planifrons* (Guinea), CI= *T. planifrons* (Côte d'Ivoire), SL= *T. planifrons* (Sierra Leone).

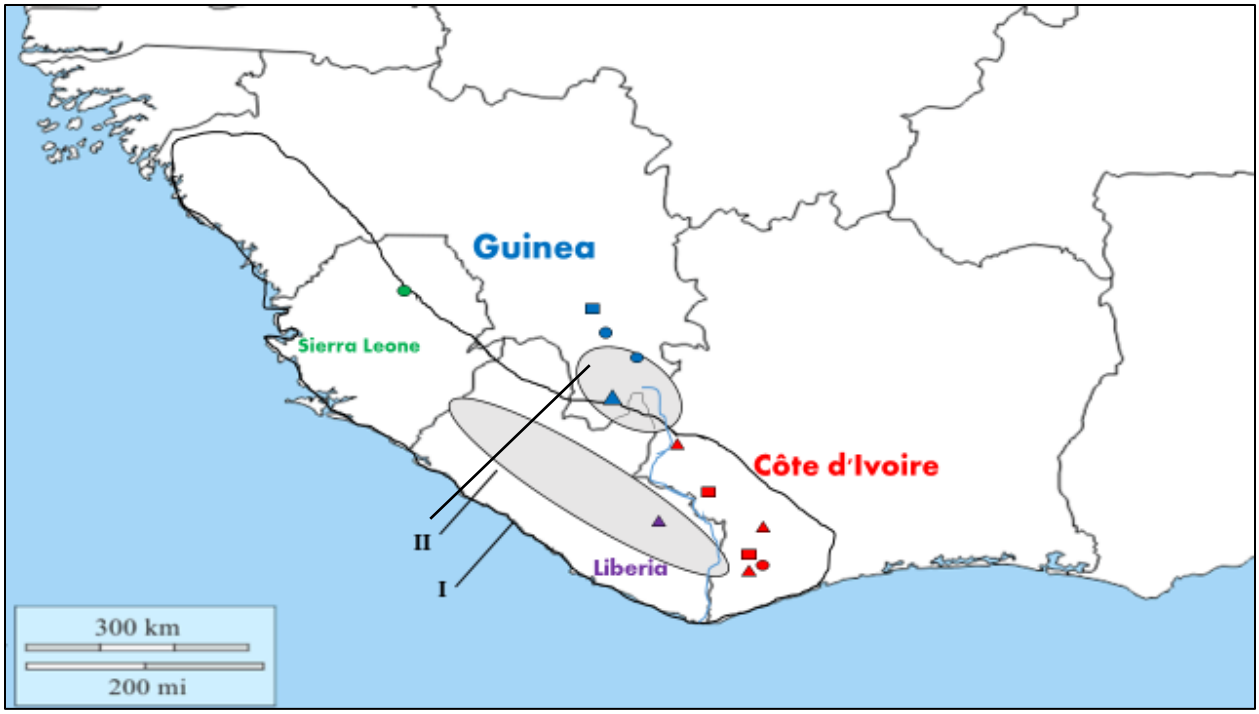

Figure 1

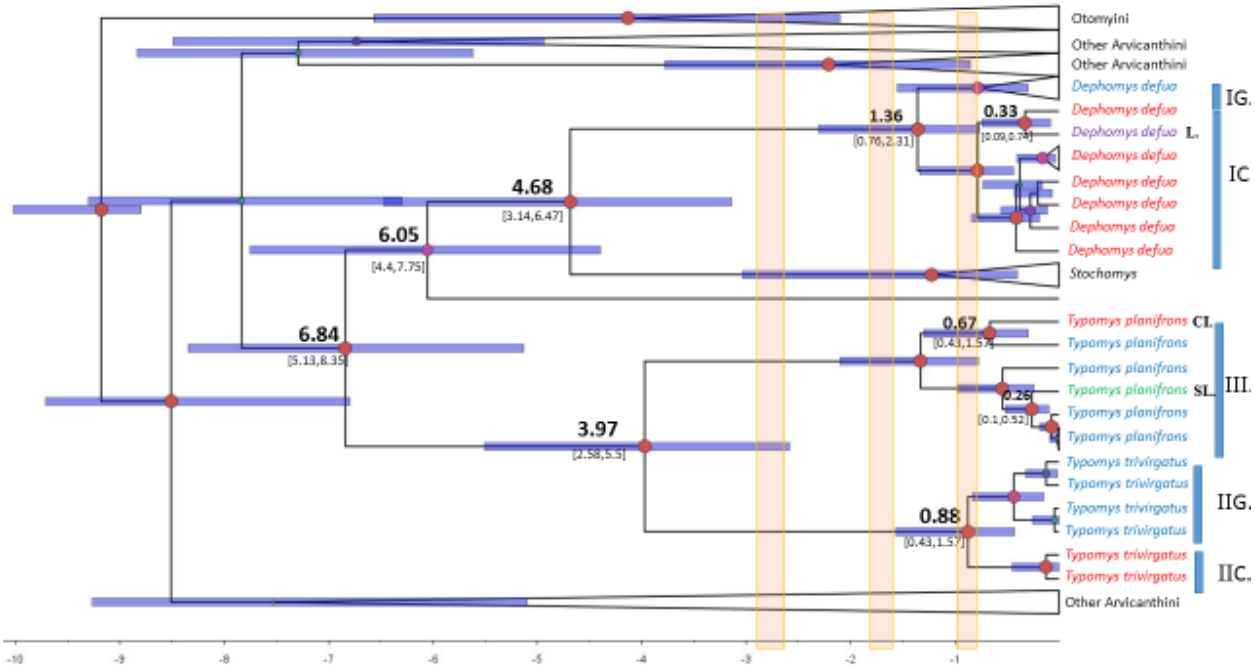

Figure 2

Table 1- GenBank accession numbers.

Species	***Cytb***	***Rbp3***
Otomys denti	EU874449	KC953428
Parotomys brantsii	AF141224	KC9534232
Golunda ellioti	AM408338	AM408332
Oenomys_hypoxanthus_1	AM408342	AM408324
Aethomys chrysophilus	AJ604526	AY326101
Pelomys fallax	DQ022382	DQ022391
Lamottemys okuensis	JQ639327	JQ639321
Grammomys gazellae	AM408345	AM408329
Grammomys surdaster	EU349747	KC953379
Mylomys dybowskii	AF141212	EU292146

Table 2- Samples I sequenced at the Ohio State University at Lima (bold) or were novel in Pradhan et al. (in review). All samples were included in DNA analysis.

Species	Tissue #	Locality	Coordinates	*Cytb*	*Rbp3*
Mylomys dybowskii	UVM2764	Guinea, Simandou	UTM 945217 N, 511148 W	**X**	
Hybomys univittatus	GA2632	Gabon, Monts Doudou		**X**	**X**
Typomys trivirgatus	UVM2825	Guinea, Simandou	UTM N 952755 W 512433	**X**	**X**
Typomys trivirgatus	P603	Guinea, Ziama		**X**	
Typomys trivirgatus	P3870	Guinea, Ziama		**X**	
Typomys trivirgatus	P3879	Guinea, Ziama		**X**	
Typomys trivirgatus	UVM1844	Côte d'Ivoire, Haute Dodo Forest	4°54’1.9"N, 7°18’57.5"W	**X**	
Typomys trivirgatus	UVM1845	Côte d'Ivoire, Cavally Forest	6°10’26.9"N, 7°47’16.6"W	**X**	**X**
Typomys planifrons	UVM1865	Guinea, Simandou	8° 31’ 53.5" N 8° 54’ 27.1" W	**X**	**X**
Typomys planifrons	UVM1866	Guinea, Simandou	8° 31’ 53.5" N 8° 54’ 27.1" W	**X**	**X**
Typomys planifrons	UVM2727	Guinea, Simandou	UTM 944965 N, 511023 W	**X**	**X**
Typomys planifrons	UVM2505	Guinea, Mt. Béro	8°08’ 11.9"N, 8°34’ 18.5”W	**X**	**X**
Typomys planifrons	UVM2506	Guinea, Mt. Béro	8°08’ 11.9"N, 8°34’ 18.9”W	**X**	
Typomys planifrons	UVM1843	Côte d'Ivoire, Haute Dodo Forest	4°54’1.9"N, 7°18’57.5"W	**X**	**X**
Typomys planifrons	UVM2588	Sierra Leone, Seli River	9° 05' 34.9"N 11° 43' 24.6"W	**X**	**X**
Dephomys defua	UVM2502	Guinea, Diéké	7°29’ 40"N, 8°49’ 54”W	**X**	**X**
Dephomys defua	UVM2503	Guinea, Diéké	7°29’ 40"N, 8°49’ 54”W	**X**	
Dephomys defua	UVM1838	Côte d'Ivoire, Haute Dodo Forest	4°54’1.9"N, 7°18’57.5"W	**X**	
Dephomys defua	UVM1839	Côte d'Ivoire, Haute Dodo Forest	4°54’1.9"N, 7°18’57.5"W	**X**	
Dephomys defua	UVM1840	Côte d'Ivoire, Haute Dodo Forest	4°54’1.9"N, 7°18’57.5"W	**X**	**X**

Dephomys defua	UVM1841	Côte d'Ivoire, Cavally Forest	6°10’26.9"N, 7°47’16.6"W	**X**	**X**
Dephomys defua	UVM1842	Côte d'Ivoire, Cavally Forest	6°10’26.9"N, 7°47’16.6"W	**X**	
Dephomys defua	ROM_100487	Côte d'Ivoire, Tai Forest		X	X
Dephomys defua	MNHN_R24062	Côte d'Ivoire		X	X
Dephomys defua	MNHN_R24148	Côte d'Ivoire		X	X
Dephomys defua	UVM2882	Liberia, Putu Range		**X**	
Stochomys longicaudatus	CM_108122	Cameroon, Baro		X	
Stochomys longicaudatus	CM_90877	Gabon, Cap Esterias		X	X
Stochomys longicaudatus	UVM1558	Ghana, Volta Region	7°07’N, 0°36’E	**X**	**X**